



## Technical Resources

Protocols

Vectors

Tools

Citations

FAQs

eNotes

Promega Notes

Cell Notes

Guides

MSDS

Publications

Contact Us

Literature Request

New Products

All Applications

## FAQs

### RNasin® Plus RNase Inhibitor

See Technical Resources for more information.

1. What is RNasin® Plus RNase Inhibitor?
2. Will RNasin® Plus RNase Inhibitor work better than native or Recombinant RNase Ribonuclease Inhibitor?
3. How is RNasin® Plus RNase Inhibitor more resistant to oxidative stress than recombinant native RNasin®?
4. What are the characteristics of RNasin® Plus RNase Inhibitor?
5. How can I use the characteristics of RNasin® Plus RNase Inhibitor to better protect my RNA template?
6. How do the characteristics of RNasin® Plus RNase Inhibitor relate to higher temperature reverse transcriptase (RT) reactions?
7. What applications are compatible with RNasin® Plus RNase Inhibitor?

#### 1. What is RNasin® Plus RNase Inhibitor?

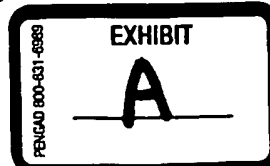
RNasin® Plus RNase Inhibitor is a recombinant mammalian RNase inhibitor expressed as a soluble protein in *E. coli*. Through natural amino acid diversity RNasin® Plus RNase Inhibitor has increased resistance to oxidation when compared to the human protein.

#### 2. Will RNasin® Plus RNase Inhibitor work better than native or Recombinant RNase Ribonuclease Inhibitor?

RNasin® Plus RNase Inhibitor is naturally more resistant to oxidative stress, increasing overall efficacy. As an added feature RNasin® Plus displays continued inhibition at higher temperatures. Also, RNasin® Plus is expressed by *E. coli* as a soluble protein allowing easy purification by a combination of ion exchange and hydrophobic interaction chromatography. No direct affinity chromatography is required. This new process yields >90% pure protein with no *E. coli* RNase carryover. However, the mechanism of inhibition remains the same for RNasin® Plus and native or Recombinant RNasin® Inhibitor. The mechanism is an inhibition of eukaryotic RNases via stoichiometric 1:1 noncovalent binding of the RNasin® Plus RNase Inhibitor to an RNase.

#### 3. How is RNasin® Plus RNase Inhibitor more resistant to oxidative stress than native or recombinant RNasin®?

Two cysteines in the human protein have been identified as especially sensitive to oxidation and react by forming a disulfide that can block the active site of the inhibitor (1). RNasin® Plus RNase Inhibitor, through natural amino acid diversity, lacks the ability to form a blocking disulfide.



**4. What are the characteristics of RNasin® Plus RNase Inhibitor?**

During development of the RNasin® Plus RNase Inhibitor Promega scientists discovered continued inhibition of RNases even above the normal denaturation temperature of the RNasin® Plus molecule. A mixture of RNasin® Plus and a pure RNase, like RNase A heated to at least 70°C for 15 minutes, and the RNase A activity does not return after cooling to normal temperatures, such as those used in the RT step of RT-PCR. It has been demonstrated to work in this manner with a complex mixture of RNases in a rat liver protein extract (Sigma Cat.# L1380). Rat liver is known to contain ribonucleases (2). No detectable RNase activity, as determined by RT-PCR, is observed when a mixture of rat liver RNases and RNasin® Plus RNase Inhibitor is heated for 15 minutes followed by the addition of 100ng or 10ng of template RNA and incubation for an additional hour at 37°C.

**5. How can I use the characteristics of RNasin® Plus RNase Inhibitor to protect my RNA template?**

Many protocols, including those for ImProm-II™ Reverse Transcription System require an initial thermal denaturation of the RNA template of interest in the presence of reverse transcription primers for 5–10 minutes at 70°C followed by a quick chill step denatures secondary structure in the RNA template, allowing greater sensitivity for PCR. In light of the new activities identified for RNasin® Plus RNase Inhibitor, it can now be added at this step to protect the RNA template during thermal denaturation. RNases that were present during the thermal denaturation will be inactivated; however, more RNase inhibitor should be added during full RT reaction assembly in the event additional exogenous RNases are inadvertently added to the reaction from pipette components or other sources.

**6. How do the characteristics of RNasin® Plus RNase Inhibitor relate to high temperature reverse transcriptase (RT) reactions?**

The characteristics of RNasin® Plus RNase Inhibitor allow you to set up your high temperature first-strand synthesis reactions and take them to reverse transcriptase reaction temperatures above 50°C. This gives researchers RNase protection when transcribing RNA templates with high secondary structure.

**7. What applications are compatible with RNasin® Plus RNase Inhibitor?**

RNasin® Plus RNase Inhibitor has been tested in RT-PCR and is compatible with such as AMV, M-MLV and ImProm-II™ Reverse Transcriptases or Taq and T7 DNA Polymerases. RNasin® Plus RNase Inhibitor has also been tested and found to be compatible with quantitative, real-time RT-PCR reactions in a TaqMan® Assay. The new inhibitor is also compatible with the Riboprobe® System for in vitro transcription using the T3, T7 RNA Polymerase. RNasin® Plus RNase Inhibitor can also be used with Wheat Germ and Rabbit Reticulocyte Lysate for in vitro translation from an RNA template, as in the TNT® Wheat Germ and TNT® Reticulocyte Lysate System for coupled in vitro transcription/translation.

**References**

1. Kim, B.M., Schultz, L.W. and Raines, R.T. (1999) Variants of ribonuclease inhibitor resist oxidation. *Protein Sci.* **8**, 430–4.
2. Zhao, W. et al. (1998) Ribonucleases from rat and bovine liver: Purification, spectroscopic and structural characterization. *Biochim. Biophys. Acta* **1384**, 55–65.